

What is Claimed is:

1. A recombinant slow-growing mycobacterium comprising at least one mycobacterial gene containing an unmarked mutation.
2. The recombinant slow-growing mycobacterium of Claim 1 wherein the mutant mycobacterial gene comprises a deletion, addition, substitution or point mutation.
3. The recombinant slow-growing mycobacterium of Claim 2 wherein the mycobacterial gene is a gene that encodes an enzyme essential in the biosynthetic pathway of a nutrient, structural component or an amino acid.
4. The recombinant slow-growing mycobacterium of Claim 3 wherein the mycobacterium is auxotrophic for lysine.
5. The recombinant slow-growing mycobacterium of Claim 4 wherein the mycobacterial gene is lysA.
6. The recombinant slow-growing mycobacterium of Claim 5 which is selected from the group of *M. bovis BCG*, *M. tuberculosis*, and *M. leprae*.
7. A method for preparing the recombinant slow-growing mycobacterium of Claim 1, comprising:
 - (a) introducing a vector into a slow-growing mycobacterium, said vector comprising a selectable marker, a counterselectable marker, and an unmarked mutant mycobacterial gene;
 - (b) selecting for primary recombinants incorporating the selectable marker;
 - (c) culturing the primary recombinants incorporating the selectable marker;
 - (d) selecting for secondary recombinants that have lost the counterselectable marker; and
 - (f) isolating the secondary recombinants comprising the desired unmarked mutant mycobacterial gene.
8. The method of Claim 7, wherein the vector is a suicide plasmid.

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9. The method of Claim 8, wherein the selectable marker confers antibiotic resistance and the counterselectable marker is one of rpsL, pyrF, and sacB.
10. The method of Claim 9, wherein the counterselectable marker is sacB.
11. The method of Claim 7, wherein the recombinant slow-growing mycobacterium is auxotrophic for lysine.
12. The method of Claim 11, wherein the mycobacterial gene is lysA.
- ~~13.~~ A vaccine that comprises (i) a recombinant slow-growing mycobacterium comprising at least one mycobacterial gene containing an unmarked mutation and (ii) a physiologically acceptable carrier.
14. The vaccine of Claim 13, wherein the unmarked mutation is a deletion, addition, substitution or point mutation.
15. The vaccine of Claim 14, wherein the slow-growing mycobacterium is auxotrophic for lysine.
16. The vaccine of Claim 15, wherein the gene is lysA.
17. The vaccine of Claim 16, wherein the slow-growing mycobacterium is selected from *M. bovis BCG*, *M. tuberculosis*, and *M. leprae*.
18. A method of treating or preventing tuberculosis in a subject comprising administering the vaccine of Claim 17 in an amount effective to treat or prevent tuberculosis in the subject.